

CLAIMS

1. A method of detecting the presence of a meiotic spindle in a mammalian oocyte comprising the following steps:

producing a plurality of intensity images of the oocyte using polarized-light optics,

5 calculating from said plurality of intensity images an image of the retardance at a plurality of points in the oocyte, wherein said retardance image provides a sensitivity of 3 nm of retardance or less, and

determining the presence or absence of a meiotic spindle in the oocyte based on the presence or absence of a spindle structure in the retardance image.

2. A method of detecting the location of a meiotic spindle in a mammalian oocyte comprising the following steps:

producing a plurality of intensity images of the oocyte using polarized-light optics,

10 calculating from said plurality of intensity images an image of the retardance at a plurality of points in the oocyte, wherein said retardance image provides a sensitivity of 3 nm of retardance or less,

locating a spindle structure in the retardance image, and

determining the location of the meiotic spindle in the oocyte based on the location of the spindle structure in the retardance image.

20 3. A method of determining the morphological characteristics of a meiotic spindle in a mammalian oocyte comprising the following steps:

producing a plurality of intensity images of the oocyte using polarized-light optics,
calculating from said plurality of intensity images an image of the retardance at a
plurality of points in the oocyte, wherein said retardance image provides a sensitivity of 3 nm of
retardance or less,

- 5 locating a spindle structure in the retardance image, and
 determining the morphology of the meiotic spindle based on the morphology of the
spindle structure in the retardance image.

4. A method of evaluating the likelihood of fertilization of a mammalian
oocyte comprising the following steps:

 producing a plurality of intensity images of the oocyte using polarized-light optics,
 calculating from said plurality of intensity images an image of the retardance at a
plurality of points in the oocyte, wherein said retardance image provides a sensitivity of 3 nm of
retardance or less,

 determining at least one of the presence, location, and morphological characteristics of a
meiotic spindle in the oocyte, based on at least one of the presence, location, and morphological
characteristics of a spindle structure in the retardance image of the oocyte, and

 evaluating the likelihood of fertilization based on at least one of the presence, location,
and morphological characteristics of the meiotic spindle.

20

5. A method of evaluating the developmental potential of a mammalian
oocyte for one of successful development, implantation, and pregnancy, comprising the

following steps:

producing a plurality of intensity images of the oocyte using polarized-light optics,

calculating from said plurality of intensity images an image of the retardance at a plurality of points in the oocyte, wherein said retardance image provides a sensitivity of 3 nm of retardance or less,

determining at least one of the presence, location, and morphological characteristics of a meiotic spindle in the oocyte, based on at least one of the presence, location, and morphological characteristics of a spindle structure in the retardance image of the oocyte, and

evaluating the developmental potential based on at least one of the presence, location, and morphological characteristics of the meiotic spindle.

6. A method of increasing the fertility rates in mammalian oocytes undergoing in-vitro fertilization procedures, comprising the following steps:

for each oocyte being considered as a candidate for in-vitro fertilization procedures, producing a plurality of intensity images of the oocyte using polarized-light optics,

calculating from said plurality of intensity images an image of the retardance at a plurality of points in the oocyte, wherein said retardance image provides a sensitivity of 3 nm of retardance or less,

determining at least one of the presence, location, and morphological characteristics of a meiotic spindle in the oocyte, based on at least one of the presence, location, and morphological characteristics of a spindle structure in the retardance image of the oocyte, and

evaluating the likelihood of fertilization based on at least one of the presence, location,

00001598-062506

and morphological characteristics of the meiotic spindle.

7. A method as in claim 6 further comprising

using the evaluation of fertilization likelihood in each candidate oocyte to determine its

5 fitness for use in subsequent procedures including at least one of fertilization, culturing, and
implantation.

8. A method of predicting aneuploidy in a mammalian oocyte, comprising

the following steps:

producing a plurality of intensity images of the oocyte using polarized-light optics,

calculating from said plurality of intensity images an image of the retardance at a
plurality of points in the oocyte, wherein said retardance image provides sensitivity 3 nm of
retardance or less,

determining at least one of the morphological characteristics and total retardance of a
meiotic spindle in the oocyte, based on at least one of the morphological characteristics and total
retardance of a spindle structure in the retardance image of the oocyte, and

evaluating the likelihood of aneuploidy in the oocyte based on at least one of the
morphological characteristics and the total retardance of the meiotic spindle.

9. A method of improving in-vitro fertilization procedures performed on an

oocyte, comprising the following steps:

handling the oocyte in a sample holder,

09801598-0625075

placing the sample holder on an inverted microscope with a sample stage and at least one objective,

viewing the oocyte with the inverted microscope and the objective,

outfitting the microscope stage with a thermostatically controlled heater,

5 outfitting said objective with a lens warmer, and

adjusting the thermostatically-controlled heater and the lens warmer to produce a nominal temperature at the oocyte with a temperature variation of less than 3 degrees C.

10. A method as in claim 9 wherein said nominal temperature is about 37° C.

11. A method for enucleation of mammalian oocytes, comprising the following steps:

providing an oocyte and enucleation apparatus, wherein said enucleation apparatus has a distal end that is brought into contact with the oocyte,

using a microscope to provide a viewing field that comprises the oocyte and the distal end of the enucleation apparatus,

producing a plurality of intensity images of the viewing field using polarized-light optics,

developing from said plurality of intensity images an image of the retardance at every point in the viewing field, wherein said retardance image provides a sensitivity of 3 nm of

20 retardance or less,

locating a spindle structure in the retardance image,

determining the location of a meiotic spindle within the viewing field from the location of

0981598-06250
#05290-86510860

the spindle structure in the retardance image, and

using the location of the meiotic spindle to effect at least one of removal of a spindle from an oocyte, and verification that a spindle has been removed from an oocyte.

5 12. A method for nuclear transfer in mammalian oocytes, comprising the following steps:

 providing an oocyte and enucleation apparatus, wherein said enucleation apparatus has a distal end that is brought into contact with the oocyte,

 using a microscope to provide a viewing field that comprises the oocyte and the distal end of the enucleation apparatus,

 producing a plurality of intensity images of the viewing field using polarized-light optics,
 developing from said plurality of intensity images an image of the retardance at every point in the viewing field, wherein said retardance image provides a sensitivity of 3 nm of retardance or less,

 locating a spindle structure in the retardance image,
 determining the location of a meiotic spindle within the viewing field from the location of the spindle structure in the retardance image, and

 using the location of the meiotic spindle to effect at least one of implantation of a spindle into an oocyte, and verification that a spindle has been implanted into an oocyte.

09881598.062504